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WHOLE-BODY EXPOSURES TO A PHOSPHORIC ACIDS AEROSOL: II. FOOD/WATER/WEIGHT EFFECTS IN WILD RODENT AND AVIAN SPECIES

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*Separate inhalation-chamber studies were conducted to assess acute/subacute food intake (g), water intake (ml), and body weight (g) effects of four whole-body phosphoric-acids-aerosol exposures in black-tailed prairie dogs (*Cynomys ludovicianus*); subacute effects of two exposures were studied in rock doves (*Columba livia*). A 95% red phosphorus/5% butyl rubber (RP/BR) mixture was burned to produce the aerosol. Each study involved (1) 3 RP/BR target concentration groups (0.0, 1.0, and 4.0 mg/L), (2) 24 prairie dogs or doves (8/group), with gender included as a factor in each study, and (3) a successive 3-phase paradigm (2 d preexposure; 4 and 2 d of ~80 min/d exposures to RP/BR for prairie dogs and rock doves, respectively; and 6 d postexposure). Results showed that in-chamber atmospheres were uniform and acceptable for all exposures. No prairie dogs died, but 1 male rock dove died on d 3 postexposure to 4.0 mg/L aerosol. Concentration and gender main effects were significant for the acute (2 h out-of-chamber) food intake, water intake, and weight change of prairie dogs, with aerosol-exposed and male rodents showing decrements. Gender and day main effects were also significant for the subacute (23 h/d) variables in prairie dogs; females weighed less than males, and reduced food/water/weight was evident for all animals during the 4 exposure days and first 3 postexposure days. For rock doves, subacute gender and day main effects, plus concentration × day and concentration × gender × day interactions, characterized the data. A transitive relationship was evident among RP/BR aerosol conditions (0.0 < 1.0 < 4.0 mg/L) and mean decreased food intakes on the exposure days (d2 < d1). Enhanced postexposure water replenishment by female versus male doves exposed to 4.0 mg/L RP/BR aerosol was a main finding. Results are explained based upon a temporal model of phosphoric acid caused ulcers/edema. Effects are compared to prior evidence for albino rats, prairie dogs and rock doves; these are also discussed relative to certain human health and ecotoxicological literature.*

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INTRODUCTION

A 95% red phosphorus and 5% butyl rubber (RP/BR) product composes a smoke grenade under development by the U.S. Army (Burton et al., 1982; Yon et al., 1983). Detonation of these grenades ignites small RP/BR particles, which are dispersed during the explosion to produce a dense white smoke that obscures troop/armor movements. The smoke consists largely of phosphoric (H_3PO_4) and polyphosphoric acids (e.g., $H_4P_2O_7$, $H_5P_3O_{10}$), with traces of hydrogen (H_2) and carbon monoxide (CO) (Brazell et al., 1984). Inhalation-chamber studies involving animal/bird exposures to this smoke afford potential human health and ecotoxicological tests of acid aerosol end points.

Recently, the potential health hazards of acid aerosol inhalation have been reviewed (Environmental Protection Agency, 1989). Numerous inhalation-chamber studies involving diverse species of animals (e.g., dog, guinea pig, monkey, rabbit, rat) have focused on the effects of mainly sulfuric acid (H_2SO_4), nitric acid (HNO_3), ammonium sulfate [$(NH_4)_2SO_4$], ammonium bisulfate (NH_4HSO_4) and sulfur dioxide (SO_2). Outcomes of H_2SO_4 exposure vary among species: (1) no effects in guinea pigs given 5-d exposures to 10 mg/m^3, with some mortality at 30 and 100 mg/m^3 concentrations (Cavender et al.. 1977); (2) ulcers of the larynx and upper trachea, epithelial ulceration, edema, and inflammatory infiltration in mice given 10- and 14-d exposures to 170 and 140 mg/m^3 aerosol, respectively (Schwartz et al., 1977); and (3) increased pulmonary resistance and decreased dynamic compliance in guinea pigs given 1-h exposures to $\geq$14.6 mg/m^3 (Silbaugh et al., 1981). Additionally, aerosol atmospheric and intraspecies factors have been shown to influence results. Larger particle aerosols (>1.0 μm) are more effective than smaller ones (<0.5 μm) at inducing respiratory/pulmonary effects (Phalen, 1984); further, young animals, plus older animals of questionable health, are more likely to display respiratory/pulmonary effects (Environmental Protection Agency, 1989).

Measurements of food intake, water intake, and body weight have often been used as secondary indicators of sublethal toxicosis in animals (Annau, 1972; Benitz, 1970; Brown, 1988). Exposures to aerosols and pollutants produce morphological/physiological changes that, in turn, cause "general malaise" (Brown, 1988). Unique patterns of food/water/weight shifts have been linked with specific inhalants and dosages (Lewkowski et al., 1979).

Regarding RP/BR aerosol effects on food/water/weight variables, Aranyi et al. (1988) reported findings from 2 chronic RP/BR-aerosol studies involving 2.25 h/d, 4 d/wk, 13 wk inhalation-chamber exposures in albino rats. Exposure concentrations were 300, 750, and 1200 mg/m^3 for Study 1 and 50, 180, and 300 mg/m^3 for Study 2. Observed effects included 10.8% mortality for rats exposed to 1200 mg/m^3 concentrations, decreased body weight and food consumption for rats exposed to $\geq$750 mg/m^3 concentrations, decreased pulmonary bactericidal activity for rats exposed to $\geq$300 mg/m^3, and increased bronchiolar fibrosis for rats exposed to $\geq$180 mg/m^3 aerosols.

In a series of subacute studies allied with the present research, Shumake et al. (1989, 1992) reported numerous pharmacotoxic sign/consummatory effects in prairie dogs and rock doves during a 28-d period following multiple RP/BR aerosol exposures. Prairie dogs showed no mortality after one to four ~80-min/d exposures to 2.0, 4.0, and 6.0 mg/L RP/BR concentrations, whereas rock doves showed 26% (10 : 24 M & 1 : 18 F) mortality within 8 d after similar exposures to 3.0 and 6.0 mg/L aerosols. Respiratory difficulties were pronounced in rock doves, and both species displayed altered or lost vocalization. Several complex exposure × gender × day interactions characterized the water intake and body weight data of these species (i.e., no food intake measurements recorded). Prairie dogs showed 1 or 2 d of suppressed body weight after multiple exposures to 6.0 mg/L aerosol; rock doves displayed 22 d of reduced weight after 2 exposures to this concentration. Postexposure water consumption for both species was unchanged initially, but was elevated from d 10 through 28 postexposure (delayed hyperdipsia).

This paper describes separate inhalation chamber studies of RP/BR aerosol-induced food intake, water intake, and body weight effects in black-tailed prairie dogs and rock doves. The research extends portions of Shumake et al. (1989) to include food intake measurements and acute post-exposure effects, particularly in prairie dogs. Acute ($\leq$2 h out-of-chamber) and/or subacute [2 d preexposure, 4 (prairie dogs) or 2 (rock doves) d exposure, and 6 d postexposure] differences in food/water/weight variables were examined. The alternative hypothesis (H_1) in each study stated that multiple, daily exposures to "target concentrations" of 1.0 or 4.0 mg/L RP/BR aerosol would cause transitive acute and subacute decreases in food intake, subacute increases in water intake, and concomitant changes in body weight of experimental relative to control (0.0 mg/L) rodents/birds. Inhalation of H_3PO_4 is thought to irritate mucosal/esophageal tissues, thereby causing hypophagia (due to esophageal pain) and delayed hyperdipsia (due to fluid loss from potential acid-produced ulcers/edema), with body weight reflecting these food and water intake shifts.

METHODS

Detailed descriptions of animal care procedures, aerosol exposure systems, and aerosol atmospheric measurements are contained in Sterner (1993); a synopsis of these procedures is provided here.

Prairie Dogs/Rock Doves

The black-tailed prairie dog and rock dove were chosen for this research because of their (1) widespread distribution, (2) representativeness of wild mammalian and avian orders, and (3) adaptability to laboratory procedures.

The black-tailed prairie dog is a large, diurnal, herbivorous, burrowing, colonial ground squirrel that inhabits the short-grass prairieland of the North American Great Plains (Jones et al., 1983). The rodents are tan in color, measure ~30–42 cm in length, and weigh ~0.5.–1.5 kg (Jones et al., 1983). Behaviorally, prairie dogs are gregarious, but territorial; average "towns" (~130 animals) cover about 7 hectares and are comprised of ~23 coteries—1 adult male, with 3–4 females plus 4–6 juveniles (Hoogland, 1985).

The rock dove is the common feral pigeon. Adult birds measure ~30–36 cm in length and weigh between 300 and 600 g (Goodwin, 1983; Reilly, 1968). This species inhabits almost all of North America south of 50° latitude. Behaviorally, pigeons cluster in flocks of <10 to >300 birds, scavenging grains and other foods discarded by humans—hence the large urban populations. Numerous scientific data exist for this species.

Twenty-four black-tailed prairie dogs (12 M, 12 F) and 24 rock doves (11 M, 13 F) were used. Prairie dogs were captured at Buckley Air National Guard Base, Aurora, Colo. (permits 87-0047 and 88-0047). Rock doves were purchased from a local supplier who netted the birds in the Denver metropolitan area. Upon capture/delivery, the rodents/birds were sexed and quarantined (14 d) in temperature- (18 ± 5°C) and light-controlled (12 : 12 h light : dark) communal areas or cages. The capture, quarantine, maintenance, and test of all rodents/birds adhered to provisions of the "Guide for the Care and Use of Laboratory Animals" (Department of Health, Education, and Welfare, 1978) and the *Code of Federal Regulations* (CFR) 9 (Parts 1, 2, and 3) concerning animal welfare (Department of Agriculture, 1985).

Although precapture histories are unknown, neither the prairie dogs nor the rock doves were used in any other research at the Denver Wildlife Research Center prior to these studies. Throughout the research, prairie dogs were maintained on Purina Rabbit Checkers (Purina Mills, Inc., St. Louis, Mo.) and water ad libitum, with fresh cabbage provided at least 3 times/wk. Doves were provided Purina Pigeon Checkers (Purina Mills, Inc., St. Louis, Mo.) and water ad libitum during the study, with cracked corn and grit included during holding and quarantine periods.

Aerosol Exposure Systems

Two separate inhalation-chamber systems were used to expose the rodents/birds to either RP/BR aerosol or equivalent durations of filtered air: the RP/BR aerosol system, and the filtered air system (Sterner et al., 1991; Sterner, 1993). The inhalation chambers were commercial stainless steel units (91.5 × 91.5 × 91.5 cm) with autoclave doors (Bertke and Young, Cincinnati, Ohio). Each chamber had 3 shelves containing 4 stainless steel wire mesh animal cages (30.5 × 30.5 × 30.5 cm).

Filtered/humidified air (250 L/min) was piped via flexible PVC tubing (10.16 cm ID) to a custom glass pipe junction (burn chamber) where RP/BR was extruded under hydraulic pressure (300–1000 psi) and ignited. The 95% RP/5% BR product was prepared by the Bio-organic Analysis Section, Analytical Chemistry Division, Oak Ridge National Laboratory, Oak Ridge, Tenn. From the burn chamber, the RP/BR aerosol or filtered air was drawn through ~7.5 m of rigid and flexible stainless steel pipe (5.6 cm ID) surrounded by a cooling jacket to the inhalation chamber—no cooling jacket was used with the filtered air system. Laminar flow of aerosol/air was assumed to occur from apex to base of each chamber. The aerosol/air was exhausted from the base of each chamber via rigid polyvinyl chloride (PVC) pipe (5 cm ID) and moved to a 7-bank, DX-grade coalescent filter unit (Balston Filter Products, Lexington, Mass.), which removed aerosol and associated contaminants from the chamber exhaust (Holmberg et al., 1985); no DX-grade filter was used with the filtered air system. Finally, the "scrubbed air" flowed to the vacuum source (Dayton Electrical Mfg., Co., Chicago) via flexible PE tubing (5 cm ID) and exited the systems (building) through ceiling vents.

Chamber Atmosphere Measurements

Periodic measurements of the RP/BR aerosol and filtered air atmospheres included (1) aerosol mass (gravimetric analysis using borosilicate glass filters), (2) H_3PO_4 concentration (titration analysis via a Radiometer DTS-800 multititration system, Radiometer America, Inc., Cleveland), (3) opacity (infrared diode and phototransistor device; see Higgins et al., 1978), (4) particle sizes (cascade impactor, California Measurements, Inc., Sierra Madre, Calif.), (5) respiratory/contaminant gases (industrial-hygiene tubes; Gastec Detection System, Gastec Inc., Newark, Calif.), and (6) temperature (T)/relative humidity (RH).

Table 1 presents composite median or minimum/maximum values obtained for in-chamber atmospheric variables during the prairie dog and rock dove exposures. Inspection of Table 1 shows that (1) RP/BR-aerosol mass concentrations were less than "target values" due to the approximate 20-min dilutions during "chamber fill/vent periods," (2) H_3PO_4 concentrations accounted for two-thirds to three-fourths of the aerosol compositions, (3) particle sizes were respirable ($\leq$1.0 μm) and typical of smoke aerosols ($\leq$0.85 μm; Phalen, 1984), (4) respiratory gases were sufficient for normal pulmonary function, and (5) contaminant gases were within acceptable limits.

Procedures

Prairie Dogs Prairie dogs were maintained in individual Plexiglas boxes (42.2 × 43.2 × 31.7 cm). Custom-made, 2.54-cm-high floor inserts consisting of 1.27-cm^2 gridded stainless steel wire supported each prairie dog above an absorbent paper. The sides and top of each box were ventilated with 20–30 holes of 0.95 cm diameter. Food was available in a semicircular stainless steel cup (6.67 cm radius × 5.08 cm deep) attached to the

TABLE 1. Composite Median (Minimum/Maximum) RP/BR Aerosol and Filtered-Air Chamber Values for Selected Atmospheric Variables During the Prairie Dog and Rock Dove Studies

Variable	Target concentration		
	0.0 mg/L	1.0 mg/L	4.0 mg/L
Aerosol			
Mass (mg)	2.9 (−20.0–19.2)	66.7 (57.1–91.4)	294.9 (248.9–338.7)
Mass concentration (mg/L)	0.13 (0.0–0.2)	0.82 (0.4–1.1)	3.60 (1.5–4.0)
H_3PO_4 (mg)	ND	46.0 (40.1–50.0)	214.4 (83.5[a]–238.9)
H_3PO_4 concentration (mg/L)	ND	0.57 (0.4–0.6)	2.64 (1.0–2.9)
H_3PO_4 as percent of mass	ND	70.1 (60–74)	73.7 (51.6–73.7)
Particle size			
Mass median aerodynamic diameter (μm)	NM	(0.2–0.4)	(0.2–0.8)
Respiratory gases			
O_2 (%)	(21–22)	(21–23)	(18–22)
CO_2 (ppm)	(484–847)	(484–968)	(605–968)
Contaminant gases			
CO (ppm)	ND	(10–22)	(15–30)
PH_3 (ppm)	ND	ND	ND
C_6H_{14} (ppm)	ND	ND	ND
Exposure/chamber			
Length of exposure (min)	(80–106)	(80–92)	(80–86)
Temperature (°C)	(21–25)	(20–24)	(21–26)
Relative humidity (%)	(50–63)	(52–67)	(48–82)

Note. ND, not detected. NM, not measured.

[a]This value was a lone outlier; it occurred during a RP/BR burn having several extinguished flames.

inside wall (~8 cm above the floor). Water was provided in a 200- and 100-ml commercial drink tube (Oasis Pet Products, Napa, Calif.) affixed to the outside of each box so that the spout protruded inside through a 2-cm-diameter hole.

The boxes were located in an isolated room (2.7 × 3.7 × 2.8 m) on standard laboratory tables. Light was provided by 4 overhead fluorescent fixtures; a 12:12-h light-dark schedule (0600–1800, 1800–0600 h, respectively) was maintained throughout each study. Modal (minimum-maximum) T and RH for the housing room was 20°C (19–24°C) and 33% (21–88%), respectively.

Following 5–10 d of acclimation to housing conditions, 8 prairie dogs within each of 3 replications were ranked by body weight within gender class (4 M and 4 F per replication). The weights of the males and females ranged from 1109 to 1371 g and 958 to 1363 g, respectively. Animals were assigned "quasi-randomly" in sets of three or two (heaviest to lightest) to one of the three groups. The term "quasi-randomly" refers to the constraints imposed on actual random assignments within replications. Only eight boxes were used; hence, to balance the prairie dogs by sexes among the aerosol groups, unequal numbers of animals had to be assigned to groups for each replication. Assignments of prairie dogs to the 0.0, 1.0, and 4.0 mg/L RP/BR aerosol groups were replication 1-2 (1 M, 1 F), 3 (1 M, 2 F) and 3 (2 M, 1 F); replication 2-3 (2 M, 1 F), 2 (1 M, 1 F) and 3 (1 M, 2 F); and replication 3-3 (1 M, 2 F), 3 (2 M, 1 F) and 2 (1 M, 1 F) animals, respectively.

Daily measurements of food intake (mg), water intake (ml), and body weight (g) were obtained during a 1-h maintenance session (0800–0900 h); these reflected intakes/weights for the preceding 23-h period (0900–0800 h). Briefly, the housing room was entered and the water level of each drink tube was recorded. Sets of 4 rodents each were removed from their boxes and placed in metal cans (17.8 cm high × 15.2 cm diameter) having perforated plastic lids. Each rodent was weighed and confined to a can for about 20 min. Body weight and food intake (spillage corrected) were determined using a Mettler 3600 Electronic Balance (Mettler Instrument Corp. Highstown, N.J.). Water intake was assessed by recording the change in water levels (meniscus) of each graduated drink tube (ml) between successive maintenance sessions. Food cups were weighed, cleaned, refilled, reweighed, and values were recorded. Each housing box was cleaned and the absorbent paper replaced. The prairie dogs were then replaced in their respective boxes and the procedure repeated for the remaining four animals. Finally, the water bottles of all rodents were refilled, and the meniscus within each bottle was checked and recorded.

During the exposure phase, procedures differed slightly. The daily maintenance procedures remained the same (0800–0900 h); however, at approximately 0900 h, rodents in the 1.0 mg/L group were removed from their housing boxes and transported to the inhalation-chamber area. Rodent identifications were verified via implanted transponders, and each prairie dog was placed into a randomly assigned exposure cage in the inhalation chamber. After all animals were loaded into the chamber, the door was closed and the ~80-min exposure was conducted. Upon completion of the exposure session, rodents were removed, reidentified, reweighed and returned to their housing boxes. After 2 h had elapsed, these prairie dogs were again reweighed and their food and water intake recorded (acute measurements). Exposures were always conducted in the sequence 1.0, 4.0, and 0.0 mg/L RP/BR aerosol; this was done to avoid buildup of acid residues from high concentration burns (chamber flushed between each exposure) and to select the longest daily exposure time for use with the filtered air controls. Typically, prairie dogs were out of their housing boxes at 0830–1100 h, 1130–1400 h, and 1430–1700 h for the 1.0, 4.0, and 0.0 mg/L groups, respectively.

Rock Doves Procedures were basically the same as those described for prairie dogs. Main exceptions were that (1) modal (minimum–maximum) T and RH for the housing room were 21°C (16–23.5) and 50% (34–90), (2) water was provided in a 100-ml graduated tube with an "open reservoir" protruding into each housing box, (3) weight ranges of doves at assignment were 257–387 g for males and 293–354 g for females, and (4) specific "quasi-random" assignments of doves to 0.0, 1.0, and 4.0 mg/L groups were replication 1-2 (1 M, 1 F), 3 (1 M, 2 F), and 3 (2 M, 1 F); replication 2-3 (2 M, 1 F), 2 (1 M, 1 F) and 3 (0 M, 3 F); and replication 3-3 (1 M, 2 F), 3 (2 M, 1 F) and 2 (1 M, 1 F) doves.

Designs/Statistical Analyses

Figure 1 is a schematic of the research design used with each species. Each study involved 3 separate groups of prairie dogs and rock doves exposed to 0.0 (filtered air), 1.0, and 4.0 mg/L RP/BR aerosol. A 3-phase, sequential paradigm was used: a 2-d preexposure phase, a 4- or 2-d exposure phase for prairie dogs and rock doves, respectively, and a 6-d postexposure phase. The sex of prairie dogs was balanced among groups (4 M and 4 F/group); however, the gender of rock doves was unbalanced among groups (i.e., 4 M, 4 F for the 0.0 and 1.0 mg/L groups and 3 M, 5 F for the 4.0 mg/L group). This gender misassignment with rock doves resulted from an error in sex determination for one bird using a cloacal examination technique (Miller and Wagner, 1955); definite sexing of each bird was performed following euthanasia and necropsy at the end of the study. Finally,

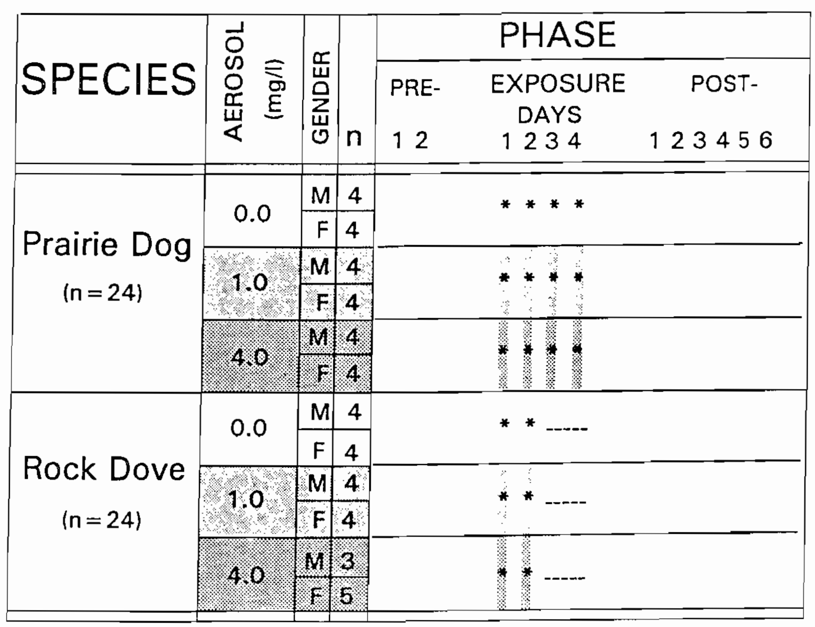

FIGURE 1. Schematic of the acute (2-h out-of-chamber) and subacute (2 d pre/4 d exposure/6 d post) designs with prairie dogs and the subacute (2 d pre/2 d exposure/6 d post) design with rock doves.

data were collected during 3 successive replications of 8 prairie dogs/rock doves each, but this factor was omitted from analyses due to limited degrees of freedom in the designs—experimental error was assumed equal across replications.

All data were analyzed using the Statistical Analysis System (SAS Institute, Inc., 1985). Analyses of variance (ANOVAs) for designs having missing data were computed using the General Linear Model (PROC GLM Program); designs with complete data were computed using PROC ANOVA. Significant sources of variance were further assessed using post hoc Duncan multiple range tests (Duncan, 1955); the .05 alpha level was used with all tests.

Prairie Dogs The amount of food consumed (g), amount of water consumed (ml), and change in body weight (g) for the 2-h out-of-chamber sessions (acute) were analyzed separately to assess relative differences among the 0.0, 1.0, and 4.0 mg/L RP/BR aerosol groups immediately after exposure. Each of these variables was analyzed as balanced 3 (concentration) × 2 (gender) × 4 (day) ANOVAs, where day was a repeated measures factor (Winer, 1971). Subacute effects of daily food intake (g), water intake (ml) and body weight (g) were analyzed as 3 (concentration) × 2 (gender) × 12 (day) factorials, with day treated as a repeated measures factor (Winer, 1971). Subacute food/water intake variables for the exposure phase involved sums of the 2-h (acute) and remaining 21-h (housing box) measurements for each rodent/bird.

Rock Doves Subacute measurements of daily food intake (g), water intake (ml), and body weight (g) for doves were analyzed as 3 (concentration) × 2 (gender) × 10 (day) factorial ANOVAs, where day was a repeated measures factor (Winer, 1971).

RESULTS

Mortality was negligible; no prairie dogs died, but 1 male rock dove died on day 3 of the postexposure phase (4.0 mg/L group). Descriptions of signs displayed by these species following diverse RP/BR aerosol exposure schedules are given in Shumake et al. (1992).

Prairie Dogs

Acute Effects There were significant concentration and gender main effects for the acute analyses. Figure 2 presents bar graphs of the concentration main effects for food intake ($F = 5.33$, df = 2/18, $p \leq .015$), water intake ($F = 12.01$, df = 2/18, $p \leq .0027$) and weight change ($F = 8.53$, df = 2/18, $p \leq .0025$) during the 2-h out-of-chamber sessions over the 4 exposures for prairie dogs. Mean (±SD) food intake, water intake, and weight change values for the 0.0, 1.0, and 4.0 mg/L groups were food intake (g) = 7.9 (±6.1)/2.8 (±3.1)/2.3 (±2.5), water intake (ml) = 33.5 (±27.1)/7.9 (±11.5)/7.4 (±12.4) and weight change (g) = 15.25 (±22.6)/–8.2

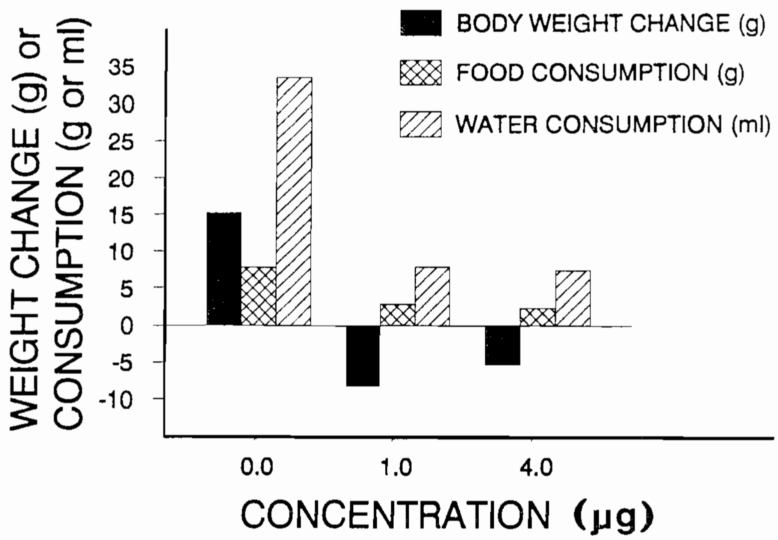

FIGURE 2. Bar graphs of the mean food intake, water, intake and body weight change of prairie dogs in the 0.0 (filtered air), 1.0, and 4.0 mg/L RP/BR aerosol groups during the 2-h periods immediately following the 4 exposures—the acute concentration main effects.

(±13.5)/–5.3 (±13.5). Post hoc Duncan multiple range tests confirmed that the 0.0 mg/L (filtered air) group mean food/water/weight variables were significantly greater than those for the 1.0 and 4.0 mg/L groups, but that means for the RP/BR aerosol groups did not differ from each other.

The gender main effects for 2-h out-of-chamber data were also significant for food intake (F = 12.01, df = 1/18, $p \leq .001$), water intake (F = 9.73, df = 1/18, $p \leq .0029$), and change in body weight (F = 9.67, df = 1/18, $p \leq .003$). Results indicated that female prairie dogs displayed greater food and water consumption, as well as gained significantly more weight, during these 2-h acute sessions than male prairie dogs. Mean (±SD) food intake, water intake, and weight change were 5.3 (±4.7) versus 3.4 (±4.9) g, 20.8 (±24.1) versus 11.8 (±18.8) ml and 4.8 (±21.1) versus –3.6 (±17.8) g (loss), respectively, for females compared to males.

Subacute Effects Analyses of daily body weights for the 12 d of the study yielded gender (F = 14.46, df = 1/18, $p \leq .0013$) and day (F = 5.28, df = 11/198, $p \leq .0001$) main effects. Day main effects were also significant for the 23-h food intake (F = 35.54, df = 11/198, $p \leq .0001$) and water intake (F = 12.89, df = 11/198, $p \leq .001$) variables.

Regarding gender, mean weights of male prairie dogs were 14.2% more than those of females (1254.2 ± 97.0 vs. 1098.1 ± 90.1 g, respectively). This simply confirmed that the males weighed more than the females upon assignment and that this weight difference persisted across the phases of the study.

Figure 3 is a line graph of the day main effects for mean food/water/

weight variables of the 24 prairie dogs on each of the 12 study days. Posthoc Duncan mean comparisons for body weight revealed that the rodents (1) weighed significantly more on preexposure d 2 than d 1 (1185.8 ± 129.4 vs.1175.2 ± 127.7 g) and (2) weighed significantly less relative to preexposure d 2 (1185.8 ± 129.4) on each of the 4 successive exposure (1169.8 ± 127.2, 1166.0 ± 121.0, 1168.7 ± 119.2, and 1163.9 ± 117.0 g, respectively) and (3) (1170 ± 121.6, 1176.1 ± 124.3, and 1180.0 ± 123.4 g, respectively). Means for the food and water intake variables reflected essentially this same pattern; however, consumptions of food and water were no different from preexposure by postexposure d 1.

Rock Doves

Subacute Effects Analyses of daily food intake, water intake, and body weight variables for the 10 d of this study yielded significant gender ($F = 9.56$, df = 1/18, $p \leq .0063$; $F = 7.44$, df = 1/18, $p \leq .0138$; and $F = 8.72$, df = 1/18, $p \leq .0085$, respectively) and day ($F = 9.99$, df = 9/159, $p \leq .0001$; $F = 6.46$, df = 9/159, $p \leq .0001$; and $F = 2.53$, df = 9/159, $p \leq .0097$, respectively) main effects for each variable in rock doves. Concentration × day ($F = 2.35$, df = 18/159, $p \leq .0025$) and concentration × gender × day interactions ($F = 2.63$, df = 18/159, $p \leq .0007$) were also significant for food and water intake, respectively.

The gender main effects for the mean food/water/weight variables were expected, with female doves eating 20.2% less (25.3 ± 6.1 vs. 20.2 ± 5.1

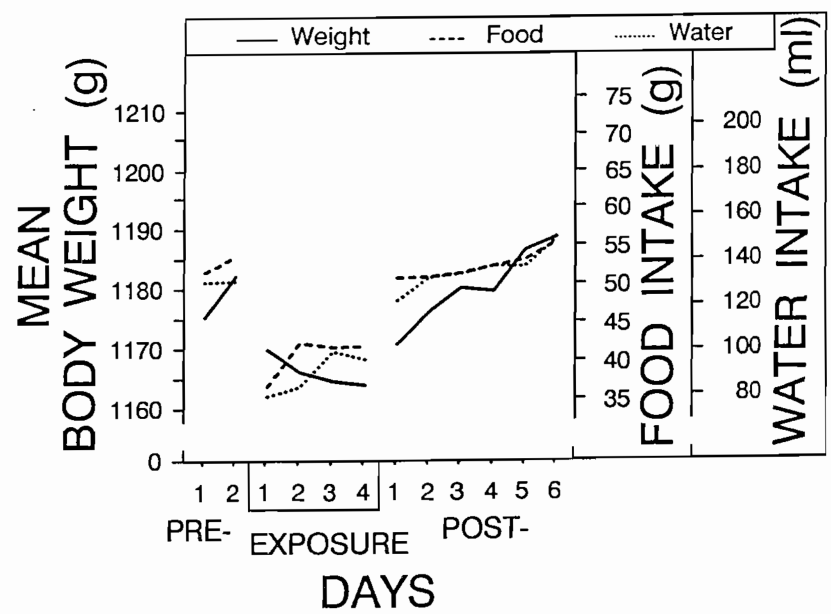

FIGURE 3. Graph of the mean daily food intake, water intake, and body weight variables for all prairie dogs (n = 24) across the 12 d of the study—the subacute day main effects.

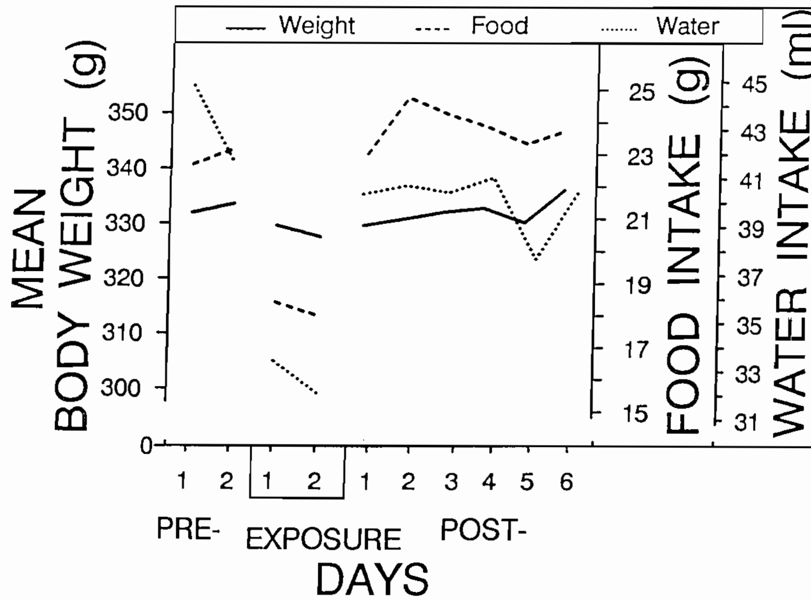

FIGURE 4. Graph of the mean daily food intake, water intake and body weight variables for all rock doves (n = 24) across the 10 d of the study—the subacute day main effects.

g), drinking 21.1% less (44.5 ± 12.7 vs. 35.1 ± 9.9 ml) and weighing 8.5% less (347.4 ± 31.4 vs. 318.0 ± 18.1 g) than male doves. These effects confirmed the relatively smaller average size of female than male doves—an inherent difference at the time of random assignment to groups.

Figure 4 is a composite line graph of the mean daily food/water/weight variables for the 24 doves on each day of the study. Post hoc Duncan multiple range tests confirmed that each of these variables were depressed during exposure days. Recovery of preexposure food intake was alleviated on d 1 postexposure, water intake was not recovered during the 6-d postexposure phase, and body weight depression was alleviated on d 4 postexposure.

A significant concentration × day interaction was found for the food intake of doves exposed to 0.0, 1.0, and 4.0 mg/L; a line graph of this interaction is shown in Figure 5. Inspection of Duncan tests showed that 4.0, 1.0, and 0.0 RP/BR aerosol-exposed doves decreased food intake 39.1%, 13.1%, and 11.3% during exposure (means ± SD of 21.8 ± 6.1 vs. 13.1 ± 6.5 g, 23.0 ± 6.7 vs. 20.0 ± 3.8 g, and 24.8 ± 9.2 vs. 22.0 ± 7.6 g between d 2 preexposure and d 1 exposure, respectively). Thus, a transitive relationship existed between RP/BR concentrations and severity of decreased food intakes (g/d) during the exposure phase, 0.0 < 1.0 < 4.0 mg/L. Postexposure recovery of food intake was rapid and involved slightly increased consumption for the 4.0 mg/L RP/BR aerosol group. Post hoc mean comparisons indicated that (1) mean food intakes for the 4.0 mg/L group on postexposure d 2–6 exceeded preexposure values, (2) the 1.0 mg/L group showed 3 d (days

1, 2, and 3 postexposure) of "hyperphagia," after which average food intake did not differ from that of preexposure, and (3) the 0.0 mg/L group matched its preexposure food intake on d 1 postexposure.

The concentration × gender × day interaction for daily water consumption was also significant in rock doves; this three-way interaction is plotted in Figure 6. Three points merit comment based upon Duncan mean comparisons. First, the daily water intakes of male birds were generally greater than those of females, a result probably due to the larger size of male doves (gender main effect). Second, subgroups of male and female doves in each RP/BR group displayed some reductions in mean water intake on the exposure days, with females in the 4.0 mg/L group and males in both the 0.0 and 4.0 mg/L groups affected greatly. Third, the male and female subgroups of doves in the 4.0 mg/L RP/BR aerosol condition showed hypodipsia and hyperdipsia relative to preexposure during d 4–6 postexposure. These males showed depressed water intakes without recovery to preexposure averages (Fig. 6), whereas females showed gradually increased water consumption with recovery exceeding preexposure means on d 4, 5, and 6 postexposure.

DISCUSSION

The H_1 is partially supported in each study. Granted, current designs do not offer definitive tests; longer subacute and chronic observations, coupled

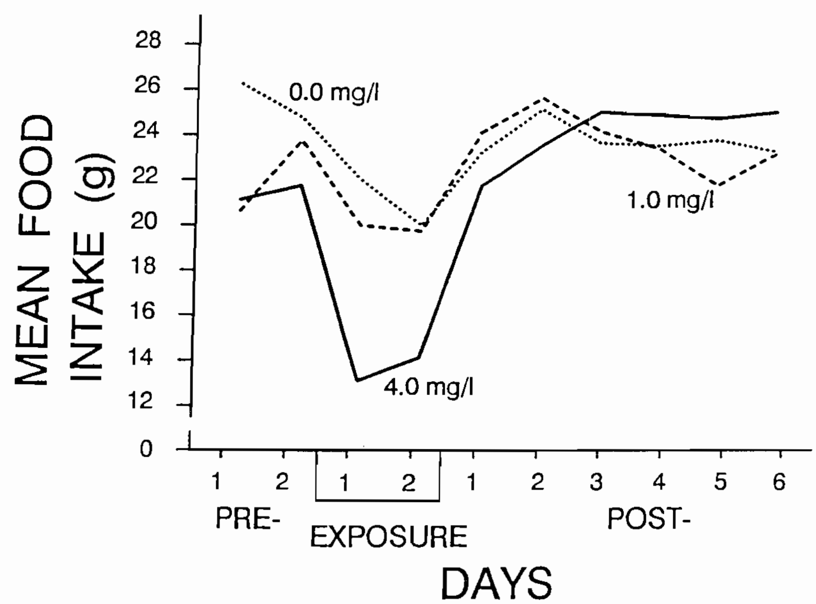

FIGURE 5. Graph of the mean daily food intakes for the 3 RP/BR aerosol groups (0.0, 1.0, and 4.0 mg/L) of rock doves (n = 8/group) across the 10 d of the study—the subacute concentration × day interaction.

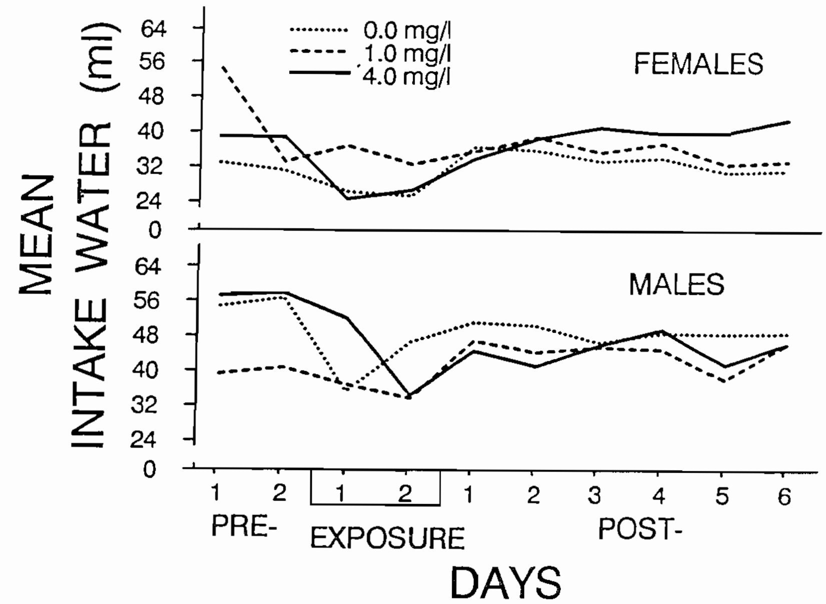

FIGURE 6. Graphs of the mean daily water intakes for female (top) and male (bottom) rock doves in each of the 3 RP/BR aerosol groups (0.0, 1.0, and 4.0 mg/L) across the 10 d of the study—the subacute concentration × gender × day interaction.

with appropriate postexposure necropsy/pathology examinations (detection of ulcers/edema), are needed to fully assess the predictions. Results suggest a model wherein acid aerosol effects occur superimposed upon "generalized stress effects" induced by handling/chamber-confinement variables. Prairie dogs display tolerance of RP/BR aerosol; rock doves are more sensitive.

Acute/Subacute Effects in Prairie Dogs

Acute findings for prairie dogs demonstrate that exposure to RP/BR aerosol causes short-term suppression of ingestive behaviors, plus concomitant weight loss. These effects do not appear to be transitive in this species for these exposure schedules. Exposure to RP/BR aerosol causes significantly greater food-/water-intake decrements and weight change (loss), but without differences between the 1.0 and 4.0 mg/L RP/BR groups.

Patterns of acute and subacute concentration, gender, or day main effects for the food/water/weight variables in both species confirm the importance of "chamber-confinement stressors" upon these body maintenance variables; however, in the absence of interaction terms the observed main effects must be attributable to factors experienced by all animals/birds. The concentration main effects for prairie dogs are the sole results for this species suggesting that RP/BR aerosol exposures produce acid irritation of mucosal/esophageal tissues that could lead to the observed hypophagia and concomitant weight loss, but this acute effect for water intake does not fit the expected fluid-replenishment prediction associated with ulcerations/edema.

Despite approximately 14% lighter weight, female prairie dogs ate and drank about twice as much food and water, plus gained almost 3 times as much weight, as males during the 2 h out-of-chamber sessions. This implies inherent differences in either stress tolerance or recovery potential between the female and male rodents. Of course, a myriad of uncontrolled factors (e.g., hormonal, genetic, morphological) could produce these results, and no unequivocal interpretation is possible. Still, gender differences have been observed in previous RP/BR aerosol studies with laboratory rats. Aranyi (1983) reported less body weight loss among groups of female than male rats given 5 daily 1-h exposures of between 1.6 and 3.0 mg/L RP/BR aerosol concentrations. Such tolerance and enhanced recoveries by females suggest important differences in the physiological/biological responses to acid aerosols.

The subacute food/water/weight differences for select days are probably indicative of joint "handling/chamber-confinement and RP/BR aerosol stress," not "RP/BR aerosol stress" per se. In the absence of RP/BR aerosol group differences across phases (concentration × day interaction), day differences must be due to factors experienced by all animals. Confinement of prairie dogs within inhalation chambers is obviously stressful—the animals urinated, defecated, barked, and bit repeatedly during the chamber loading and unloading procedures. This excitement, coupled with the unavailability of food and water during the ~2.5-h loading/confinement/unloading schedules may have depleted nutrient and fluid reserves of the animals. If unaffected by the exposures, prairie dogs would be expected to quickly replenish nutrients and fluids once food and water are available. This is exactly how the control animals (0.0 mg/L group) behaved, whereas RP/BR aerosol-exposed animals displayed short-term reductions in food intake, water intake, and weight.

Subacute Effects in Rock Doves

The concentration × day (food) and concentration × gender × day (water) interactions, coupled with gender and day main effects (food/water/weight), in rock doves offer more direct support for a temporal model of nutrient depletion/fluid loss due to acid aerosol-induced ulcerations of soft tissue. Although the presence of ulcers/edema is unconfirmed, subacute effects observed for food/water intake in doves imply that pain of swallowing food and fluid loss/replenishment probably play a key role in exposure and postexposure compensations to acid aerosol insult, with females and males showing important differences.

Whole-body exposures to 0.0, 1.0, and 4.0 mg/L RP/BR aerosol produce increasingly greater mean daily reductions in food intake by rock doves, at least during exposure days (Fig. 5). These transitive differences are suggestive of more severe laryngeal/esophageal pain from acid irritation due to increased H_3PO_4 concentrations. Burton et al. (1982) reported epiglottal ulcers and laryngeal edema in rats given single, 1-h exposure to ≥3.1 mg/L RP/BR aerosol. Shumake et al. (1992) reported no ulcers/edema in prairie dogs (or doves) after even 3 or 2 exposures to 6.0 mg/L concentrations, but necropsy and histopathology checks were performed at 28 d postexposure—healing could have occurred.

Fluid-replenishment differences between male and female birds are a key outcome of the current work. This could also be relevant to the 10 : 24 versus 1 : 18 male to female mortality ratios reported for doves exposed to diverse RP/BR aerosol regimen (Shumake et al., 1989, 1992). The subtle enhancement of water intake displayed by female doves relative to males several days after exposure concurs with a "compensation model" of greater water intake as a result of acid-caused ulcers/edema (fluid replenishment), but the absence of this effect for males implies that other gender-based factors are also involved. Shumake et al. (1989) found transitive increases in water intake for doves (also prairie dogs, for that matter) administered three, two, and one ~80-min/d exposures to 3.0 and 6.0 mg/L target concentrations of RP/BR aerosol, but this occurred between d 7 and 28 postexposure. These authors also reported greater water intake by birds in the 3.0 mg/L as compared to the 6.0 mg/L conditions—results possibly attributed to the high mortality of doves in the 6.0 mg/L RP/BR groups. No short-term (≤6 d) effects upon water intake were observed by Shumake et al. (1989).

"Handling/chamber-confinement stress" also occurs for doves—day main effects. Chamber-confinement appears to exert a major impact upon the consummatory behaviors and weight loss/recovery of wild species. Regarding these stressors, Johns et al. (1992) reported data on pulmonary function, blood chemistry, and hematology for both prairie dogs and rock doves. Although few effects were observed for prairie dogs, significant concentration × sex (gender) interactions were found for hemoglobin (Hb) and methemoglobin (MetHb) variables, and significant concentration × day interactions were obtained for carbon dioxide production (V_{CO_2}), respiratory exchange ratio (RER), metabolic rate (MR), lymphocytes, and heterophiles in doves. Female doves displayed enhanced oxygen-carrying capacity shortly following exposure to 4.0 mg/L. Roughly 35% decreased lymphocyte production (61.0 to 25.5%) and about 36% increased heterophile production (35.4 to 71.4%) characterized the preexposure to exposure-phase data. Such results confirm that the "chamber-confinement stress" accounts for certain of the acute effects reported here, and point out another basis of female dove tolerance to acid aerosols.

Comparative Toxicological Effects of RP/BR Aerosol

Prior studies of RP/BR aerosol inhalation with rats have yielded LC50 values of 2.32 and 2.46 mg/L for a single, 1-h exposure (Aranyi, 1983;

Burton et al., 1982). All prairie dogs in this study survived up to 4 daily 80-min exposures at concentrations of about 4.0 mg/L, whereas all except 1 of the rock doves survived up to 2 daily ~80 min exposures to an actual median concentration of 3.74 mg/L (Sterner, 1993).

In agreement with prior observations for laboratory rats (Aranyi, 1983), RP/BR aerosol exposure causes acute and subacute shifts in food intake, water intake, and body weight for both prairie dogs and rock doves. Aranyi et al. (1983) reported mean body weight losses in male rat groups after exposure to between 1.56 and 3.05 mg/L of RP/BR aerosol levels over 5 daily 1-h exposure sessions. Body weights of female rats were less affected by these RP/BR aerosol levels. Current results reflect similar patterns. Both of the current species displayed gender-related effects. Acute weight loss following RP/BR aerosol exposure is measurable in prairie dogs, with males losing weight and females gaining weight. Prairie dogs display a very acute (1 d) average weight loss (1.2%) following the most severe exposure schedule studied here. Conversely, rock doves show direct concentration- and exposure-related losses in weight for 1–6 d after two, 4.0 mg/L exposures. Again, male doves display relatively more weight loss than females, with earlier water intake recovery believed responsible for the shorter duration weight loss of females.

Health Effects/Ecological Implications

The "destructive effects" of acid aerosols tend to increase directly as a function of particle size (Environmental Protection Agency, 1989; Phalen, 1984). Moreover, hygroscopic action is confounded with acid concentration in whole-body animal/bird exposure studies (higher concentrations induce greater affinity of H_3PO_4 for moisture), and acid neutralization from NH_3 production (defecation and urination of rodents/birds) occurs continuously, with volume of excrement directly related to greater acid neutralization.

Present measurements show that the RP/BR aerosol mass consisted of approximately two-thirds (50–75%) H_3PO_4, with mass median aerodynamic diameters (MMADs) of <0.85 μm (Table 1)—data confirming both the acidic composition and the control exerted over particle sizes ($\leq$0.8 μm). Although hygroscopic action undoubtedly varied with concentration, data indicate that exogenous H_3PO_4 particle growth was maintained within acceptable limits for current aerosol exposures. Although no volume measurements of NH_3 production were obtained, it is doubtful that NH_3 production significantly reduced the H_3PO_4 exposure concentrations for either the 1.0 or 4.0 mg/L groups of prairie dogs and rock doves.

Researchers have estimated the concentration of an "actual RP/BR smoke cloud" to range between 0.25 and 2.50 mg/L, with mass loadings greater than 1.0 mg/L present only at the time ($\leq$1 min) of initial RP/BR release (Garvey et al., 1981). The median 0.82 and 3.60 mg/L "steady-state concentrations" actually involved in the current tests represent improbable field conditions. Additionally, despite the agitation/stress displayed by both species during handling/confinement, current measurements reflect animals/birds at rest or following minimal exertion (restrictive cage size). This further complicates the discussion of environmental implications. Prolonged exercise/flight should aggravate most RP/BR smoke-induced effects in animal/bird models. Neither species represents a "highly sensitive sentinel."

In conclusion, considerations relevant to ecological assessments of aerosols have been discussed by Novak et al. (1985). They suggest that a series of diverse data collections is needed for an "ecotoxicological or ecoepidemiological assessment" of acid aerosol effects. Such a series includes (1) characterization of the chemical and physical properties of the agent, (2) identification of sensitive biological test or response systems, (3) specification of the variables needed to quantify biological effects, and (4) conduct of the appropriate studies to generate the final ecological impact data. While the present research affords a comparative perspective on the consequences of H_3PO_4 aerosol exposure, inferences to actual impacts upon wildlife and military lands are tenuous. Appropriate field studies with resident wildlife are needed.

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